Remarks

Claims 23-35 are pending in the subject application. Applicants acknowledge that claims 27-35 have been withdrawn from further consideration as being drawn to a non-elected invention. By this Amendment, Applicants have amended claims 23, 31 and 33. Support for the amendments can be found throughout the subject specification and in the claims as originally filed. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 23-35 are currently before the Examiner. Favorable consideration of the pending claims is respectfully requested.

Claims 23-26 have been rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such as way to reasonable convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Applicants respectfully assert that there is adequate written description in the subject specification to convey to the ordinarily skilled artisan that they had possession of the claimed invention.

The Examiner asserts that the terms "any group capable of being hydrolyzed" and "a vitamin, a co-enzyme" fail to meet the written description provision of 35 USC §112, first paragraph. The terms "any group capable of being hydrolyzed", "vitamin" and "co-enzyme" have been deleted and the objection is now moot.

The Office Action argues that the term "antigen" fails to meet the written description provision of 35 USC §112, first paragraph. Applicants respectfully disagree. As indicated in *Capon v. Eshhar*, 418 F.3d 1349, 1357, 76 U.S.P.Q.2d 1078, 1084 (Fed. Cir. 2005),

> The "written description" requirement implements the principle that a patent must describe the technology that is sought to be patented; the requirement serves both to satisfy the inventor's obligation to disclose the technologic knowledge upon which the patent is based, and to demonstrate that the patentee was in possession of the invention that is claimed. *See Enzo Biochem*, 296 F.3d at 1330 (the written description requirement "is the quid pro quo of the patent system; the public must receive meaningful disclosure in exchange for being excluded from practicing the invention for a limited period of time"); *Reiffin v. Microsoft Corp.*, 214 F.3d 1342, 1345-46 (Fed. Cir. 2000) (the purpose of the written description requirement "is to ensure that the scope of the right to exclude . . . does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification"); *In*

> *re Barker*, 559 F.2d 588, 592 n.4 (C.C.P.A. 1977) (the goal of the written description requirement is "to clearly convey the information that an applicant has invented the subject matter which is claimed"). The written description requirement thus satisfies the policy premises of the law, whereby the inventor's technical/scientific advance is added to the body of knowledge, as consideration for the grant of patent exclusivity.
>
> The descriptive text needed to meet these requirements varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence. The law must be applied to each invention that enters the patent process, for each patented advance is novel in relation to the state of the science. Since the law is applied to each invention in view of the state of relevant knowledge, its application will vary with differences in the state of knowledge in the field and differences in the predictability of the science.

Additionally, the burden of showing that the claimed invention is not described in the application rests on the Patent Office in the first instance. *In re Edwards*, 568 F.2d 1349, 1354 (C.C.P.A. 1978). Moreover, the determination of what is needed to support generic claims to biological subject matter depends on a variety of factors, such as the existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or technology, the predictability of the aspect at issue, and other considerations appropriate to the subject matter. *Capon*, 418 F.3d at 1359. For example, it is unnecessary for the specification to provide a description of proteins which are already known in the prior art. *Id.* at 1357-58. Thus, Applicants respectfully submit that the rejection is in appropriately applied to the claims as it is unnecessary to provide a description of antigens known in the prior art for this application to comply with the written description rejection.

Indeed the term "antigen" is defined in the application, as published, at page 14 paragraph [0159], the antigen comprises "a killed, inactivated or attenuated pathogen, microorganism or parasite" or is "an enriched or purified polypeptide, lipid, polysaccharide, glycoprotein, glycolipid or nucleic acid antigen". In addition, an extract from the widely used University textbook Immunology (published 1992), by Janis Kuby, provides evidence as to the existing knowledge in the art at the time the invention was made. In this attached document, an entire chapter is dedicated to antigens. Antigen are discussed at page 73 where it is stated that "Antigens are substances able to induce a specific immune response. The molecular properties ultimately contribute to immune activation is central to our understanding of the immune system. [...]", and the specific section, at page 93, "Viral

and bacterial antigens" provides many different examples of antigens. Thus, it is respectfully submitted that the as-filed specification and claims comply with the written description requirement of 35 U.S.C. § 112, first paragraph and reconsideration and withdrawal of the rejection is respectfully requested.

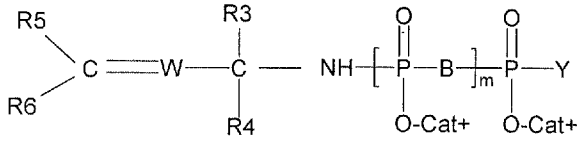
Claims 23-26 have been rejected under 35 U.S.C. §112, second paragraph, as indefinite. Applicants respectfully assert that the claims as filed are definite; however, the claims have been amended to attend to the cancellation of non-elected subject matter and the other issues noted in the Office Action. Accordingly, withdrawal of the rejection under 35 U.S.C. § 112, second paragraph, is respectfully requested.

Claims 23, 25 and 26 are rejected under 35 U.S.C. § 102(b) as anticipated by Tsuhako *et al.* (1981). The Office Action indicates that Tsuhako *et al.* discloses a compound according to the instant Formula (I) wherein R is a branched C_4 alkyl group substituted by a carboxylic group, m is 3, B is O and Y is O^-Cat^+. The Office Action also states that Tsuhako *et al.* discloses the compound prepared with valine and the sodium salt of cyclo-tetraphosphate tetrahydrate in water, a pharmaceutically acceptable carrier and implicitly in the form wherein Cat^+ is sodium. Applicants respectfully assert that the Tshuako *et al.* reference does not anticipate the claimed invention. For example, the compounds described in Figure 3 in Tsuhako *et al.* do not have a double bond in their alkyl portion of the compound. Rather, these compounds contain an aliphatic substituted carbon chain. Accordingly, reconsideration and withdrawal of the rejections under 35 U.S.C. § 102(b) is respectfully requested.

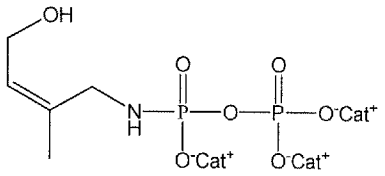
Claims 23-26 are provisionally rejected over claims 31, 32 and 49 of copending Application No. 11/817,450. A double patenting rejection of the obviousness-type is "analogous to [a failure to meet] the nonobviousness requirement of 35 U.S.C. 103" except that the patent principally underlying the double patenting rejection is not considered prior art. *In re Braithwaite*, 379 F.2d 594, 154 U.S.P.Q. 29 (C.C.P.A. 1967). Therefore, any analysis employed in an obviousness-type double patenting rejection parallels the guidelines for analysis of a 35 U.S.C. 103 obviousness determination. *In re Braat*, 937 F.2d 589, 19 USPQ2d 1289 (Fed. Cir. 1991); *In re Longi*, 759 F.2d 887, 225 U.S.P.Q. 645 (Fed. Cir. 1985). Accordingly, the analysis employed in an obviousness-type double patenting determination parallels the guidelines for a 35 U.S.C. 103(a) rejection and the

factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 U.S.P.Q. 459 (1966) are applied for establishing a background for determining obviousness under 35 U.S.C. 103.

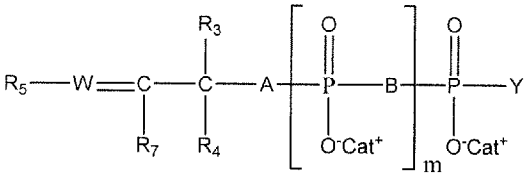
In this case, the Examiner asserts that co-pending application 11/817,450 discloses compounds of general formula:



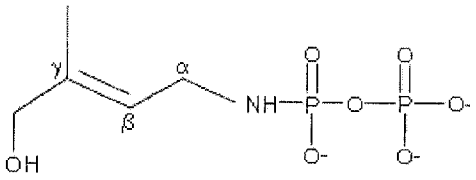
and more particularly



Applicants respectfully submit that the compounds disclosed in co-pending application 11/817,450 are patentably distinct from the compounds disclosed in the claims as amended. For example, the position of the double bond is different between the two sets of compounds. The compounds presented in currently pending claims 23-26 do not have a carbon atom in beta position (as assessed from the nitrogen atom) as defined in co-pending application 11/817,450. Rather, the compounds recited in claims 23-26 contain a substitution at the gamma position as such:



, in particular



Thus, Applicants respectfully assert that the claims as amended herein are not obvious over the claims of the cited application and the Office Action fails to establish a rationale as to why the claimed compounds should be considered obvious over the compounds disclosed in the '450 application. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position. Applicants expressly reserve the right to pursue the invention(s) disclosed in the subject application, including

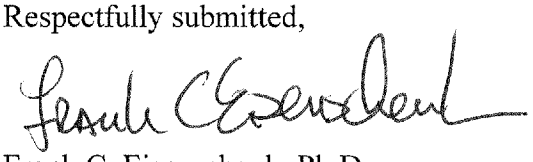
any subject matter canceled or not pursued during prosecution of the subject application, in a related application.

In view of the foregoing remarks and amendments to the claims, Applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



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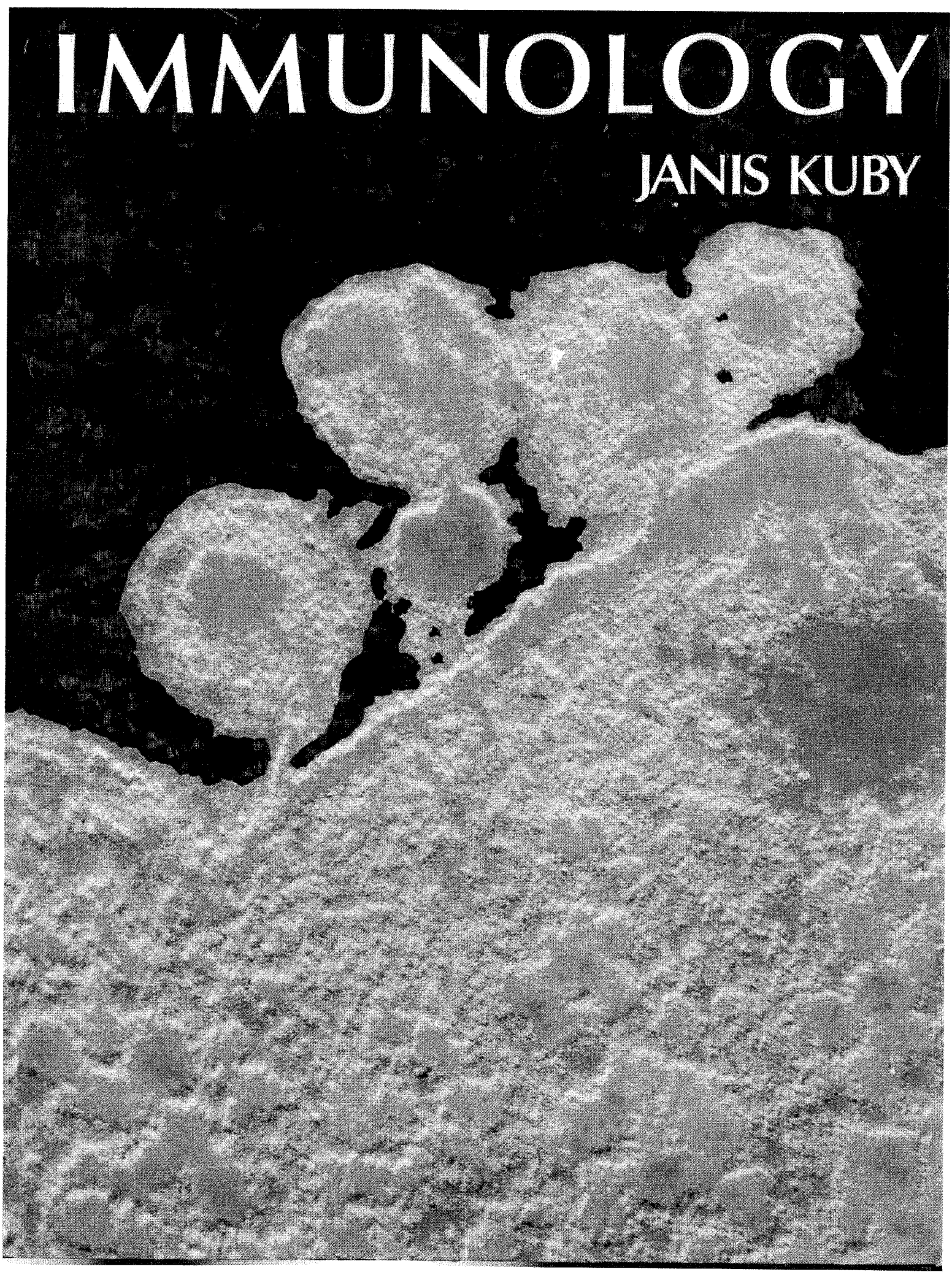
Attachment: Pages 73 and 93-97 from Immunology textbook (published 1992)

IMMUNOLOGY

JANIS KUBY



Cover illustration of AIDS viruses budding from an infected T cell was provided by L. Montagnier/CNRI, Science Photo Library.

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CHAPTER

4

Antigens

Antigens are substances able to induce a specific immune response. The molecular properties of antigens and the way in which these properties ultimately contribute to immune activation is central to our understanding of the immune system. Some of the molecular features of antigens recognized by B or T cells are described in this chapter. The contribution made by the biological system to immunogenicity also is explored, since it is ultimately the biological system that determines whether a molecule, capable of binding to a B or T cell's antigen-binding receptor, can thereupon induce an immune response. Fundamental differences in the way T and B lymphocytes recognize antigen determine which molecular features of an antigen are recognized by each branch of the immune system. These differences also are examined in this chapter and illustrated by typical viral and bacterial antigens.

Viral and Bacterial Antigens

The general properties of antigens discussed so far can be illustrated by a closer examination of viral and bacterial antigens. These antigens stimulate the immune response to viral and bacterial infection, thus triggering the body's most effective defense mechanism against infectious disease.

Viral Antigens

Animal viruses consist of nucleic acid (either DNA or RNA) surrounded by a protein coat, called a *capsid,* which is composed of protein subunits called *capsomers*. In simple viruses the capsomers are composed of a single protein; in more complex viruses several capsomer proteins may be present. A capsid with its enclosed nucleic acid is referred to as a *nucleocapsid*; a nucleocapsid may have helical or polyhedral symmetry. Some animal viruses are *naked,* but many have an additional lipoprotein *envelope*, which the virus acquires by modifying the host cell's plasma membrane as it leaves the cell in the process called *budding*. The complete viral particle is called a *virion* (Figure 4-14a). Protein—the principal constituent of animal viruses—is the only component of the capsid and a major component (sometimes in the form of glycoprotein) of the envelope. Proteins are also intimately associated with the viral nucleic acid as internal proteins of the nucleocapsid. Most of these proteins and glycoproteins can be recognized as immunogens by the immune system and will induce a humoral and/or a cell-mediated response.

B cells can recognize a variety of viral proteins and glycoproteins, including components of the envelope and interior components of the nucleocapsid, which may be released from infected host cells prior to complete viral assembly. The subunit structure of the capsid and repeating glycoprotein projections on many enveloped viruses provide the B cell with repeating epitopes. As discussed earlier, immunodominant B-cell epitopes tend to be residues that are accessible, hydrophilic, and mobile; thus surface sequences generated by the tertiary conformation of viral proteins function as the immunodominant B-cell epitopes. During the course of a viral infection, serum levels of antibody to envelope proteins, core proteins, and proteins associated with the viral genome all increase. These antibodies can facilitate virus clearance either by acting as opsonins to enhance phagocytosis or by activating the complement cascade leading to lysis of the enveloped viral particle. These antibodies often play a protective role by binding to viral envelope proteins or glycoproteins and preventing further infection of host cells. The presence of viral-specific antibodies is often used to determine whether an individual has been infected with a particular virus.

Although antibody is produced during a viral infection, in general a cell-mediated immune response is required for protective immunity to a virus. Both T_H and T_C cells can recognize viral proteins. T_H cells are generally class II MHC restricted. These T_H cells recognize viral proteins that have been internalized by the antigen-presenting cell, either by phagocytosis in the case of macrophages or by receptor-mediated endocytosis in the case of the B cells. After processing in the endosomal pathway, antigenic peptides will be displayed, together with class II MHC, on the membrane of these antigen-presenting cells. As mentioned already, the peptides recognized by T_H cells tend to be internal amino acid sequences that have amphipathic properties, enabling them to interact with both a class II MHC molecule and the T-cell receptor. Lymphokines produced by activated T_H cells then serve to activate either B cells or T_C cells.

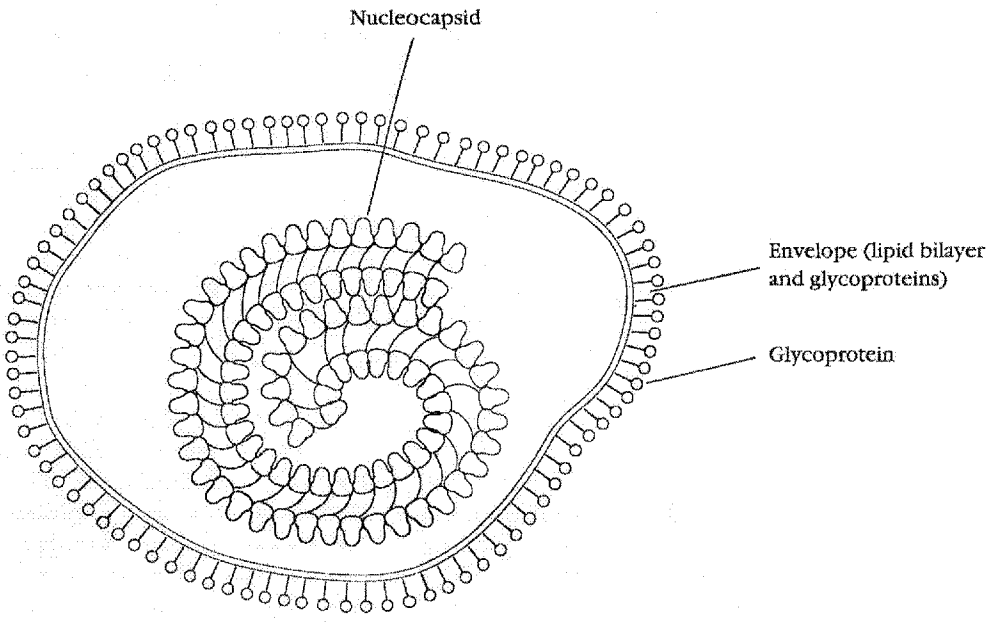
Many animal viruses are known to replicate within host cells. As viral proteins are produced within the host cell, these endogeneously produced proteins may be processed within the cytoplasm and presented together with a class I MHC molecule on the membrane of the infected host cell, inducing a T_C-cell response. The epitopes recognized by T_C cells need not be major, exposed viral components such as the envelope glycoproteins; instead, they often are internal viral proteins produced within the infected host cell. For example, a major influenza antigen recognized by T_C cells is an internal protein called nucleoprotein, which is associated with the viral RNA genome. Activation of T_C cells in response to nucleoprotein peptides appears to play an important role in the elimination of influenza-infected host cells and in recovery from the infection.

Some viruses are capable of substantial variation in the structure of their envelope glycoprotein components. Influenza virus, for example, constantly changes the amino acid sequence of its envelope glycoproteins. Either major amino acid variations (*antigenic shift*) or minor variations (*antigenic drift*) can give rise to new epitopes, allowing the virus to evade the immune system. This antigenic variation is the major cause of repeated influenza outbreaks. This process is discussed more fully in Chapter 19. Rapid changes in an envelope glycoprotein of the human immunodeficiency virus (HIV) of AIDS enable the virus to evade the immune response and thus establish a major obstacle to vaccine development, as will be seen in Chapter 21.

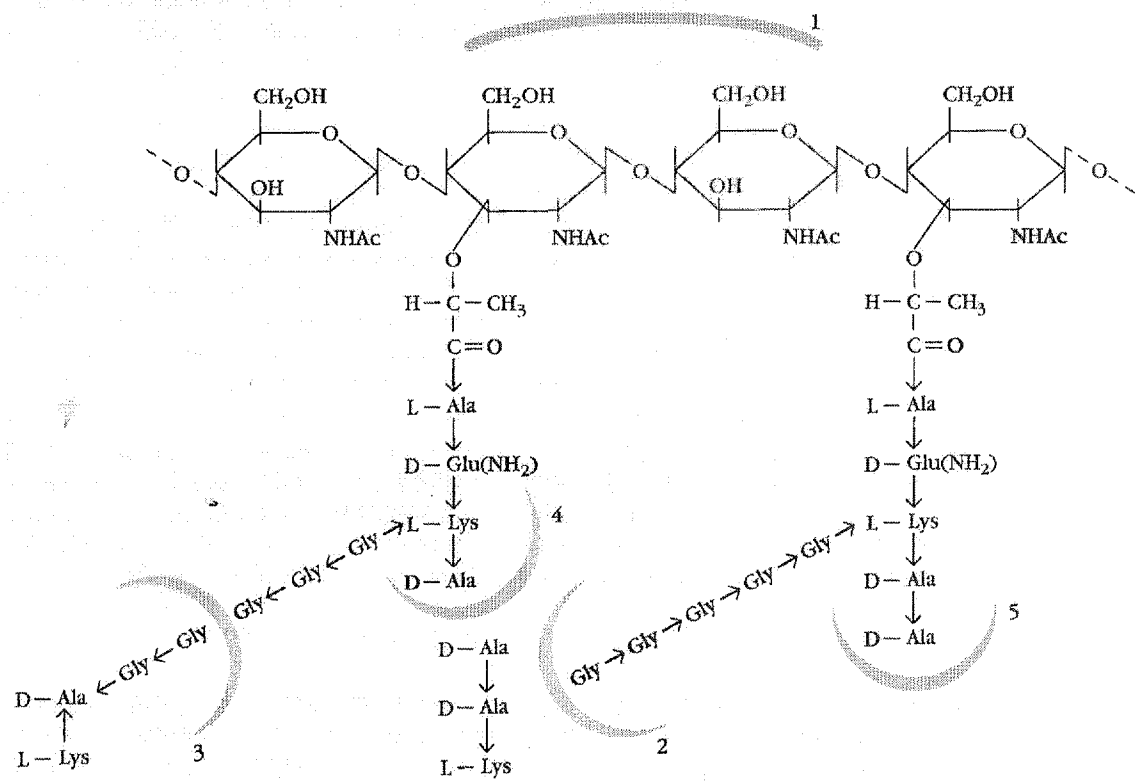
Bacterial Antigens

Bacteria are single-cell organisms, consisting typically of a membrane-bound cytoplasm, containing RNA, DNA, and enzymes, that is surrounded by a cell wall and in some cases enclosed in a capsule. Various processes (flagella, fimbriae, or pili) may protrude from the cell.

(a) Enveloped viral particle



(b) Bacterial cell-wall peptidoglycan



Although a bacterium may secrete soluble products that can serve as immunogens, the major bacterial immunogens are epitopes on surface structures.

The cell wall of so-called *gram-positive* bacteria is composed largely of peptidoglycan, a network of polysaccharides cross-linked by short peptide chains. Intercalated within the gram-positive cell wall are various proteins, polysaccharides, and teichoic acids. Structural differences in these cell-wall components generate unique epitopes, that can be recognized with antibody (Figure 4-14b). The gram-positive *Streptococci*, for example, can be grouped on the basis of antigenic differences in their cell-wall carbohydrate.

Gram-negative bacteria have a thin peptidoglycan layer covered by an outer membrane containing phospholipid, protein, lipopolysaccharide, and lipoprotein. The lipopolysaccharide (LPS) is a major antigenic component of the gram-negative cell wall. The polysaccharide side chains of LPS consist of repeating linear trisaccharides or branched tetra- or pentasaccharides; a chain can include as many as 40 repeat units. The LPS of gram-negative cell walls thus presents the immune system with accessible and multivalent epitopes on the bacterial surface, which are referred to as *O antigens*. Differences in the O-antigen epitope structure of the polysaccharide side chains can induce specific antibodies, which can be used to classify gram-negative bacteria.

The bacterial capsule is a loose polysaccharide or polypeptide layer that lies outside the cell wall. The presence of a capsule is associated with virulence because it interferes with phagocytosis. Most capsules consist of repeating sequences of two or three sugars and have molecular weights as high as 140,000 Da. The accessibility of the capsule, as well as its repeating epitope structure, allows this bacterial component to generate a significant humoral antibody response. In the case of *Pneumococci*, an estimated 4×10^6 antibody molecules can combine with the capsular epitopes expressed on a single bacterial cell. The binding of antibody to capsular epitopes provides another basis for typing bacteria. Differences in capsular polysaccharide sugars and their linkages define more than 80 pneumococcal types.

Figure 4-14 Viral and bacterial antigens. (a) Structure of an enveloped viral particle. The repeating envelope glycoproteins are B-cell epitopes, as are some internal core proteins; both can induce a humoral immune response. Internal proteins that are processed and presented on the membrane of virus-infected cells together with class I MHC molecules induce a cell-mediated response. (b) A portion of the primary structure of the bacterial cell-wall peptidoglycan showing five B-cell epitopes. [From B. Heymer, 1985, in *Immunology of the Bacterial Cell Envelope*, D. E. S. Stewart-Tull and M. Davis, eds., John Wiley and Sons.]

Mitogens

Mitogens are agents that are able to induce cell division in a high percentage of T or B cells. Unlike immunogens which activate only lymphocytes bearing specific receptors, mitogen activation is nonspecific. Mitogens are known as *polyclonal activators* because they activate many clones of T or B cells irrespective of their antigen specificity. A variety of diverse agents function as mitogens. A number of common mitogens are proteins (called *lectins*) that are derived from plants and bind sugars. Lectins recognize different glycoproteins on the surface of various cells, including lymphocytes. Lectin binding to the membrane glycoproteins often leads to agglutination, or clustering, of the cells, which is often followed by cellular activation. Some mitogens preferentially activate B cells, some preferentially activate T cells, and some activate both populations.

Three common mitogens are *concanavalin A*, or *Con A*; *phytohemagglutinin*, or *PHA*; and *pokeweed mitogen*, or *PWM*. *Con A* is a protein derived from jack bean seeds that binds to sugars containing α-D-mannose or α-D-glucose. Con A is a tetramer, with each of the four monomer units containing a carbohydrate binding site. The molecule is therefore able to crosslink glycoproteins on the surface of cells. Con A is a T-cell mitogen. *PHA* is a protein derived from kidney beans that is specific for glycoproteins containing *N*-acetylgalactosamine. Like Con A, it too is a tetramer which is able to crosslink glycoproteins on the surface of cells. PHA also functions as a T-cell mitogen. *PWM* is derived from pokeweed. It binds to di-*N*-acetylchitobiose and is mitogenic for both T and B cells.

Not all mitogens are lectins. The lipopolysaccharide, or *LPS*, component of the gram-negative bacterial cell wall functions as a B-cell mitogen. The mitogenic activity of LPS is due to its lipid A moiety which is thought to interact with the plasma membrane, resulting in a cellular activation signal through, as-yet-unknown mechanisms.

An unusual group of polyclonal activators, known as *superantigens*, are among the most potent T-cell mitogens known. Superantigens were named because of their ability to activate all T cells expressing common sequences in their T-cell receptors, irrespective of their specificity for antigen/MHC. Unlike T-cell epitopes that bind to the cleft of the MHC and are recognized by the T-cell receptor, superantigens appear to recognize residues outside the antigen binding cleft of the MHC and T-cell receptor (Figure 4-15). The superantigen thus binds simultaneously to the T-cell receptor and to the MHC molecule and activates large numbers of T cells. Included among the superantigens are the staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin (TSST1) produced by the gram positive bacterium

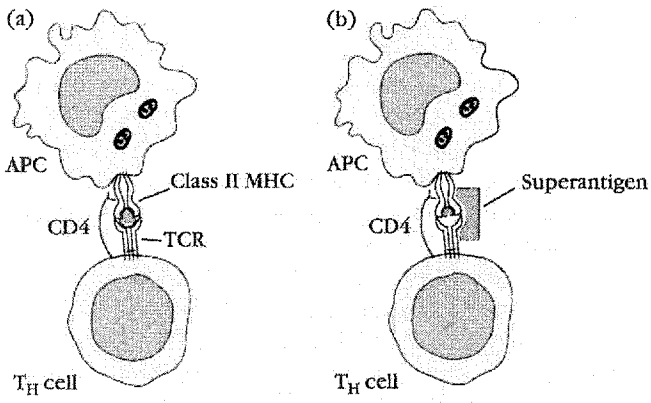


Figure 4-15 (a) Normally T-cell activation occurs after a T cell recognizes both peptide and MHC. (b) Superantigens are thought to bypass the conventional T-cell activation sequence. The superantigen is thought to bind simultaneously to the class II MHC molecule and to the Vβ chain of the TCR. This interaction enables T cells to be activated by MHC molecules bearing peptides for which the T cell is not specific.

Staphylococcal aureus. These toxins appear to activate large numbers of T_H cells by crosslinking the T-cell receptors with any class II MHC molecule expressed on an antigen-presenting cell. Estimates are that one out of every five T cells can be activated by SEs, resulting in the release of abnormally high levels of cytokines. The high levels of cytokines released can lead to shock and death, seen most dramatically in tampon-related toxic shock syndrome caused by the TSST1 superantigen. We will discuss these superantigens more fully in Chapters 11 and 19.

Summary

1. Immunogenicity is the ability of an antigen to induce an immune response within either the humoral or the cell-mediated branch of the immune system. Antigenicity is the ability of an antigen simply to interact specifically with free antibody and/or with antigen-binding receptors on lymphocytes. B cells and T cells recognize small sites called antigenic determinants, or epitopes, on a complex immunogen.

2. The foreignness, molecular size, chemical composition and complexity, and degradability of a substance influence its immunogenicity. In addition, several properties of the biological system that an antigen encounters affect its immunogenicity; these include the genotype of the recipient animal, the immunogen dose and route of administration, and the presence or absence of adjuvants.

3. The size of B-cell epitopes—those epitopes recognized by membrane-bound antibody and free antibody—is determined by the size of antibody's binding site. B-cell epitopes tend to be amino acid sequences within an antigen that are accessible, usually hydrophilic, and mobile. Sequential B-cell epitopes consist of contiguous amino acid residues along the polypeptide chain, whereas nonsequential B-cell epitopes, also called conformational determinants, are formed from noncontiguous segments of the polypeptide chain that are brought into proximity by the three-dimensional folding of a protein.

4. T-cell epitopes—those epitopes recognized by T-cell receptors—tend to be slightly larger than B-cell epitopes and generally consist of internal amino acid sequences that are hydrophobic or more commonly amphipathic. T-cell epitopes are revealed to the immune system by antigen processing, in which the protein is fragmented into small peptides that interact with class I MHC or class II MHC molecules; the resulting peptide-MHC complexes are then displayed on the surface of altered self-cells or antigen-presenting cells. The immunodominant T-cell epitopes are determined in part by the selective interactions of particular processed peptides with particular MHC molecules.

5. Haptens are small molecules that can bind to antibodies but cannot by themselves function as immunogens. The study of haptens has allowed immunologists to learn about the structural basis of antibody specificity.

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Study Questions

1. Indicate whether each of the following statements is true or false. If you think a statement is false, explain why.
   a. Most antigens induce a polyclonal response.
   b. A large protein antigen generally can combine with many different antibody molecules.
   c. A hapten can stimulate antibody formation but cannot combine with antibody molecules.
   d. MHC genes play a major role in determining the degree of immune responsiveness to an antigen.
   e. T-cell epitopes tend to be accessible amino acid residues that can interact with the T-cell receptor.
   f. B-cell epitopes are often nonsequential amino acids brought together by the tertiary conformation of a protein antigen.
   g. Both T_H and T_C cells recognize antigen that has been processed and presented with a MHC molecule.
   h. Each MHC molecule binds a unique peptide.
   i. Internal viral proteins of the nucleocapsid are not likely to be immunogenic because they are not accessible to the immune system.
   j. An influenza hemagglutinin peptide shown to induce potent proliferation of T_H cells in an H-2^k haplotype mouse would be expected to induce potent T_H-cell proliferation in an H-2^d strain as well.

2. Two vaccines are described below. Would you expect either or both of them to activate T_C cells? Explain your answer.
   a. A UV-inactivated ("killed") viral preparation that has retained its antigenic properties but cannot replicate.
   b. An attenuated viral preparation that has low virulence but can still replicate within host cells.

3. You are trying to develop a vaccine to induce cell-mediated immunity to malaria and decide to screen peptides derived from the outer-coat protein of the microorganism that causes malaria for amphipathic properties. What is the rationale for this approach? What other factors must you take into consideration in developing a vaccine by this approach?

4. In the experiment Nilabh Shastri described on page 89, why did he transfect MHC genes into mouse L cells rather than into an antigen-presenting cell such as the macrophage?

5. What are the significant differences between T-cell and B-cell epitopes?